II. REMARKS

A. State of the Claims

Claims 54, 56-73, and 75-120 were pending at the time of the Action. Claims 54, 61, 69, 71-73, 80, 86, 87, 113, 114, 118, and 120 are amended in the Amendment submitted herewith. Claim 121 is added by amendment in the Amendment submitted herewith. Claims 82, 88-90, 97, and 115-117 are canceled without prejudice or disclaimer in the Amendment submitted herewith. Therefore, claims 54, 56-73, 75-81, 83-87, 91-96, 98-114, and 118-121 are currently pending.

B. The Invention

The present invention relates to methods of treating cell extracts with a combination of a reducing agent and heat, as well as kits and combinations for carrying out such methods. These methods of treating cell extracts result in the inactivation of some or all ribonucleases that may be present in the extract, thereby preventing degradation of any RNA that may be present in the cell.

Ribonucleases (RNases) are enzymes that process and degrade RNA molecules. In addition to the endogenous ribonucleases from cells and tissues, finger grease and bacteria and/or fungi in airborne dust particles are common sources of RNases in molecular biology procedures. Consequently, there is substantial potential for contamination of molecular biology samples by RNases of all forms. If present, RNases pose a significant risk of degrading RNA in numerous procedures in the field of molecular biology. It is highly desirable to eliminate or minimize this risk.

The claimed methods have advantages over previously described methods of inactivating ribonucleases in cellular extracts that employ chaotropic agents, such as guanidinium-based

compounds. Chaotropic agents can inhibit enzymes that might be used in subsequent molecular biology procedures. Therefore, RNA from cellular extracts treated with chaotropic agents must be removed from the presence of the chaotropic agent before it is employed in further enzyme-based molecular biological procedures. Such removal is usually accomplished by procedures involving washing, centrifugation to pellet the RNA, and then resuspension of the RNA in a solution that does not contain any of the chaotropic agent. Such procedures are time and labor consuming. By contrast, the presence of a reducing agent in a solution containing enzymes such as reverse transcriptase and/or DNase 1 does not result in the inhibition and/or inactivation of those enzymes. Therefore, there is no need to spend the time and labor to remove the reducing agent from the presence of the RNA in the methods of the invention.

In some specific embodiments, the invention relates to methods of preparing cDNA from cells. In such methods, the inventors have found that it is possible to employ the use of a reducing agent and heat to inactivate ribonucleases in a cellular extract and then employ the cellular extract in an appropriate protocol to produce cDNA from RNA present in the cellular extract. Further, because it is not necessary to remove the RNA from the reducing agent prior to adding enzymes such as reverse transcriptase and/or DNase 1, the methods of the invention allow for the preparation of cDNA in a single container, without laborious and time-consuming washing, centrifugation and resuspension steps.

In addition to being useful to inactivate RNases that may be known to be present in a cellular extract, the claimed methods may be used prophylactically to eliminate the risk of contamination by RNases. This is important because a molecular biologist frequently does not know whether there are RNases present in his or her sample. It is, therefore, not necessary for a molecular biology sample to contain RNases in order for the methods of the claims to be useful

in eliminating the risk of RNases. Further, it is not necessary for the methods of the invention to result in the inactivation of each and every type of ribonucleases that may exist for the methods to be of use to those skilled in molecular biology. The fact that at least some RNases may possibly be found in a cellular extract will be inactivated is sufficient to render the invention of use to the skilled practitioner. For example, treatment of a cellular extract in a manner proven to inactivate well-known ribonucleases, such as RNase A, RNase 1, and RNase T1, in order to remove the risk of RNase degradation from those ribonucleases is an important aspect of the invention.

C. The Obviousness-Type Double Patenting Rejection is Overcome

The Action entered a provisional obviousness-type double patenting rejection to the instant application in view of co-pending application Serial No. 09/160,284. Applicants submit herewith a Terminal Disclaimer over Serial No. 09/160,284. Therefore, the provisional obviousness-type double patenting rejection is overcome.

D. The Claims Are Fully Supported By Adequate Written Description in the Specification.

1. The Rejection over the Term "Reducing Agent" is Overcome.

The Action rejects claims 54, 56-60, 69-73, 75-78, 91 and 118-120 under 35 U.S.C. § 112, first paragraph, stating that the full scope of the claim term "reducing agent" is not described and/or enabled in Appellant's disclosure. The Action, page 6, beginning at line 13. The Action also cites the previous Office Action, dated November 27, 2001, which suggests that instead, the less excessively broad term "thiol-containing reducing agent" comports with the requirements of 35 U.S.C. § 112, first paragraph. The Action, page 3, line 21 through page 4, line 3.

Applicants do not agree that there is no adequate written description and enablement of the term "reducing agent" in the Specification, and further note that there is no legal requirement which could limit the claims to specific reducing agents. However, in order to speed allowance of commercially relevant subject matter, Applicants have amended the instant claims to recite that they encompass "thiol-containing" reducing agents. Applicants in no way acquiesce as to the propriety of the Action's rejection in this regard and reserve the right to prosecute claims that encompass any and all reducing agents in the future. Therefore, in view of the statements of the Action, these objections are overcome.

2. The Written Description Rejections based on Murthy *et al.* and Khesin *et al.* are Overcome.

The Action rejected claims 54, 56-73, 75-79, 91, 97, 104-114, and 118-120, and lacking written description under 35 U.S.C. §112, first paragraph, based upon Murthy *et al.* and Khesin *et al.* The basis of this rejection appears to be a position that Murthy *et al.* teaches that one specific RNase (BS-1) remains activity when treated with DTT at 37°C, and that Khesin *et al.* teaches an interferon RNase that may exhibit increased activity when treated with DTT. For all of the reasons set forth in previous responses to official actions, which are incorporated herein by reference, Applicants dispute the Action's interpretation of Murthy *et al.* and Khesin *et al.*

Despite the above, Applicants respectfully point out that the current claims do not require inactivation of any and all ribonucleases, including the RNase BS-1 of Murthy *et al.* or the interferon RNase of Khesin *et al.*

Independent claim 54 is directed to a method of treating an extract of a cell to prepare an admixture "wherein there is no detectable RNase A, RNase 1, or RNase T1 activity in the admixture." The specification clearly sets forth methods and compositions that allow for the

preparation of admixtures with no detectable RNase A, RNase 1, or RNase T1 activity, as admitted by the Action at page 6. Applicants do emphasize that claim 54 is in no way limited to only the inactivation of RNase A, RNase 1, and/or RNase T1, or to the treatment of admixtures that contain RNase A, RNase 1, and/or RNase T1. Rather, treatment of any cell extract according to the steps of the claim in order to obtain any admixture with no detectable RNase A, RNase 1, or RNase T1 activity, is within the scope of the claim.

Independent claim 73, is directed to methods of producing cDNA from one or more cells. The claimed methods involve the use of a thiol-containing reducing agent and heat, which as taught in the specification, allows for the preparation of cDNA from RNA in a cell extract without first purifying the RNA from the cell extract. Even if the Action's assertions about the teachings of Khesin *et al.* and Murthy *et al.* are true, which Applicants do not admit, those assertions are insufficient to support a written description rejection to claim 73 or its dependent claims.

In view of the above, Applicants submit that all written description rejections to the claims are overcome.

E. The Rejections Over 35 U.S.C. §112, Second Paragraph, are Overcome.

The Action entered several rejections under 35 U.S.C. §112, second paragraph, all of which are overcome for the reasons set forth below.

The rejection to claim 54, is overcome because current claim 54 sets forth that that claim is a method for achieving an admixture with no detectable RNase A, RNase 1, or RNase T1 activity in the cell. The same holds true for those claims dependent to claim 54.

Regarding the rejection to claims 86 and 87, current claims 86 and 87 have language setting forth that the kits comprise "a ribonuclease resistant artificial viral coat encapsidated RNA standard." Support for this language is found in the Specification at page 25, lines 15-19.

Regarding the rejections to claims 91-96 and 99-103, it is believed that the current claim set does not have antecedent basis issues.

Regarding claim 120, it is believed that the current claim language is appropriate.

F. The 35 U.S.C. §102(b) Rejections to Claims 80, 81, and 83-85 over Murthy *et al.* and/or Boshes *et al.* are Overcome.

The Action rejected claims 80, 81, and 83-85 as anticipated by each of Murthy *et al.* and Boshes *et al.*

Without in any way conceding as to the propriety of these rejections, Applicants would respectfully point out that the subject matter of previous claim 82 has been incorporated into present claim 80. Since previous claim 82 was dependent from previous claim 80, but not rejected over either, Murthy *et al.* or Boshes *et al.*, it is believed that current claim 80, and its dependents, are free of the Action's view of this art. This amendment to claim 80 is made without prejudice to Applicants rights to present claims of differing scope in later prosecution.

In view of the above, it is believe that the anticipation rejections are overcome.

G. The Rejection to Claims 54, 56-57, 61-72, and 97 as Obvious over Boshes *et al.* in view of Cleland *et al.* is Overcome.

The Action rejected previous claims 54, 56-57, 61-72, and 97 as obvious over Boshes *et al.* in view of Cleland *et al.* Applicants traverse.

The instant claim 54 is directed to:

A method of treating an extract of a cell comprising:
(a) obtaining at least one cell;

(b) obtaining a thiol-containing reducing agent;
(c) preparing an admixture of an extract of the cell and the thiol-containing reducing agent; and
(d) heating the admixture to a temperature and for a time required to result in the inactivation of any RNase A, RNase 1, and/or RNase T1 present in the admixture;

wherein there is no detectable RNase A, RNase 1, or RNase T1 activity in the admixture after the heating step.

Neither Boshes *et al.* nor Cleland *et al.* teach this invention.

1. Standard of Obviousness.

The M.P.E.P. outlines three requirements for establishing a *prima facie* case of obviousness, pertinent to the present rejection:

1) there must be some suggestion or motivation to combine reference teachings;

2) the prior art demonstrates a reasonable expectation of success of the invention; and

3) the combined references must teach or suggest all the claim limitations.

§ 2143. *See also, In re Vaeck*, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991). All three elements must be shown to establish a *prima facie* case of obviousness. *Vaeck* also emphasizes that both the suggestion and reasonable expectation of success must be found in the prior art, not in the Applicant's disclosure. Furthermore, the burden of showing a *prima facie* case of obviousness is on the Examiner, who must show evidence beyond merely stating that the claimed invention is obvious in light of the prior art. *See* M.P.E.P. § 2144.03; *Graham v. John Deere Co.*, 383 U.S. 1, 18 (1966). If the Examiner properly meets the burden of showing a *prima facie* case of obviousness, the Applicant may still overcome the obviousness rejection through a showing of secondary considerations such as unexpected results, long felt need, failure by others or commercial success. *See Id.* at 17-18.

2. Boshes *et al.* Does Not Support Inactivation of Ribonucleases with Reducing Agents.

As an initial point, the Boshes *et al.* reference, which was discussed at length in the Specification, does not support the use of reducing agents to inactivate ribonucleases. As set forth in the Specification, Boshes *et al.* describes an experiment in which polyribosome preparations were treated with RNase A in a solution in the presence or absence of 4 mM DTT. The treatment of polyribosomes with RNase A generated monoribosomes. Boshes *et al.* observed that polyribosomes treated with RNase A in the presence of 4 mM DTT decreased the conversion of polyribosomes to monoribosomes. Since Boshes *et al.* was working with a complex, uncharacterized protein mixture, it is unclear what may have been responsible for the decreased production of monoribosomes reported in the paper. Boshes *et al.* did not report any assay of RNase A activity, with or without exposure to DTT. The effect of DTT on the conversion of a complex mixture of polyribosomes to monoribosomes by RNase A would not suggest to one of ordinary skill that DTT, or any other reducing agent, could be used in the methods of the invention to inactivate ribonucleases.

3. The Method of Boshes *et al.* Is Ineffective at Inactivating Ribonucleases.

Data in the specification demonstrate that the method taught in Boshes *et al.* does not result in the inactivation of ribonucleases in the manner described and claimed in the application. As described in the Specification, at page 13, line 28, to page 14, line 18, Boshes *et al.* indicated that the addition of DTT (4 mM DTT at 4°C for 20 minutes) to a crude polyribosome preparation decreased the generation of monoribosomes by RNase A (10 μg/ml). However, when Appellants tested for inactivation of ribonucleases using the conditions suggested by Boshes *et al.*, they found the referenced method failed to inactivate ribonuclease. In these tests, RNase A (at 200

ng/ml, instead of the higher concentration of 10 μg/ml RNase A reported employed in Boshes *et al.*) was treated with or without 4 mM DTT in Boshes' solution A at 4°C for 20 minutes. RNase A activity was then directly assayed by incubating mouse liver total RNA with the treated RNase A at 37°C for 60 minutes. The mouse RNA was then fractionated by electrophoresis in a 1% agarose formaldehyde gel to determine the level of RNA degradation. RNA degradation was evaluated by comparing the treated RNA with untreated mouse RNA.

As measured by the assay system described in the Specification, RNase A (200 ng/ml) treated with 4 mM DTT was not inactivated using the conditions set forth by Boshes *et al.* In fact, under Boshes *et al.*'s conditions, the RNase A treated with 4 mM DTT completely digested the RNA. Therefore, the conditions reported in Boshes *et al.* are not sufficient to inhibit even 1/50 the concentration of the RNase A employed in that paper in the manner claimed in the present invention. In contrast to RNase A treated using the Boshes *et al.* method, RNase A treated at 60°C for 20 minutes in the presence of 20 mM DTT was completely inactivated, *i.e.*, the mouse RNA remained completely intact in the RNase A activity assay.

In view of the above, Boshes *et al.* does not teach a method of producing an admixture with no detectable RNase A, RNase 1, or RNase T1 activity, and cannot render present claim 54 obvious.

4. Boshes *et al.* Combined with Cleland Does Not Render the Claims Obvious.

Neither Cleland nor Boshes *et al.* contain the suggestion or motivation to combine the references for any purpose, much less for the purpose of rendering the present invention obvious. As discussed above, Boshes *et al.* does not teach a method of producing an admixture with no detectable RNase A, RNase 1, or RNase T1 activity with DTT. Therefore, any teachings of

Cleland regarding additional reducing agents are moot in view of the failure of Boshes *et al.* to teach that even one reducing agent can be used in this regard. Further even if a suggestion or motivation to combine Cleland and Boshes *et al.* could be found in the references, there would be no reasonable expectation of success in making the claimed invention.

From the points above, the combination of Boshes *et al.* and Cleland completely fails to teach or suggest the methods of the invention of present claim 54. In addition, there is no suggestion or motivation to combine the references. Therefore, none of claims 54, 56-57, 61-69 and 97 are properly rejected as obvious.

H. Conclusion

It is respectfully submitted that the rejections have been overcome and that the case is in condition for allowance.

The Examiner is invited to contact the undersigned attorney at (512) 536-3035 with any questions, comments or suggestions relating to the referenced patent application.